Role of β-Hydroxy-γ-Trimethylammonium Butyrate (L-carnitine) and Ubiquinone (CoQ_{10}) in Combating the Deteriorative Effect of Halogenated Alkanes in Liver

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ABSTRACT

Liver cirrhosis is one of the most dangerous health problems which results from various disorders that damage liver cells over time. The purpose of the current report was carried out to provide information about the liver injury induced by halogenated alkanes (CCl_4), and to determine the influence of administration of L-carnitine or/and CoQ_{10} as prophylactic agents against that injury. The study was carried out on 80 adult male albino rats divided into eight groups, 10 animals of each, as follows: four ***normal*** *groups (control, treated with L-carnitine, CoQ_{10}, and a combination of L-carnitine and CoQ_{10}) and four* ***cirrhotic*** *groups treated with CCl_4 (control, pretreated with L-carnitine, CoQ_{10}, and a combination of L-carnitine and CoQ_{10}). Liver cirrhosis was induced by s.c. injection of a single dose of CCl_4 (1 ml/kg). L-carnitine (50 mg/kg/day) was given i.p. for four successive days 24 hours before CCl_4 injection, and CoQ_{10} was given as single i.p. dose (200 mg/kg) 24 hours before CCl_4 injection. Animals were sacrificed 24 hours after CCl_4 injection, blood samples were withdrawn and liver tissue samples were homogenized. The studied parameters were: hepatic reduced glutathione (GSH), lipid peroxides, ALT and AST activities, total protein, lactate dehydrogenase (LDH) activity in both serum (with its isozymes) and liver tissues. The injection of CCl_4 produced a significant decrease in reduced glutathione content and total protein levels in serum and liver cells. However, there was a significant increase in serum ALT and AST activities, lipid peroxides, serum and hepatic total LDH and serum LDH isoenzymes. On the other hand, groups treated with L-carnitine or/and CoQ_{10} (prior to CCl_4 injection) showed improvement in most parameters when compared with cirrhotic control group. It has been concluded that L-carnitine and coenzyme Q_{10} have a pronounced prophylactic effect against liver damage induced by halogenated alkanes.*

Key words: *Coenzyme Q_{10}, halogenated alkanes, L-carnitine, lipid peroxides, LDH and reduced glutathione*

INTRODUCTION

Liver is a complex organ that is well designed for its central role in carbohydrate, protein and fat metabolism. It is the site where waste products of metabolism are detoxified. It is responsible for synthesizing and

secreting bile and synthesizing lipoproteins and plasma proteins, including clotting factors[1]. Liver is responsible for concentrating and metabolizing the majority of drugs and toxins[2].

Liver function tests are valuable in detecting impairments of liver and impending hepatitis, drug toxicity, infiltrative lesions such as tumors and certain stages of cirrhosis[3]. The determination of ALT and AST is of a real value in distinguishing hepatocellular and obstructive jaundice. They are especially important in the early diagnosis of infective hepatitis[4]. It appeared that AST activity is more sensitive reflection of chronic hepatitis and that ALT is more readily elevated by acute hepatitis[5].

GSH plays a key role in protection against tissue damage caused by oxidative stress[6] and in regulation of the immune response[7]. The GSH is an important tool in the defense mechanism against reactive oxygen species, its depletion could be attributed to its consumption by the liberated free radicals[8].

Carbon tetrachloride when administered is distributed throughout the whole body[9], with highest concentrations in liver, brain, kidney, muscle, fat and blood. The parent compound is eliminated primarily in exhaled air, while minimal amounts are excreted in the feces and urine[9]. CCl_4 intoxication, also, leads to hypomethylation of cellular components; in the case of RNA the outcome is thought to be inhibition of protein synthesis, in the case of phospholipids it plays a role in the inhibition of lipoprotein secretion. None of these processes is considered the ultimate cause of CCl_4-induced cell death; it is by cooperation that they achieve a fatal outcome, provided the toxicant acts in a high single dose, or over longer periods of time at low doses[10].

L-carnitine is 4-trimethylamino-3-hydroxybutyrate. It is synthesized chiefly in the liver and kidneys from the essential amino acid L-lysine residues in certain proteins[11]. Chronic kidney disease and some forms of liver disease may be indications for L-carnitine supplementation. Preliminary work suggested that L-carnitine can reduce fat deposits in some fatty livers[12]. Carnitine deficiencies are occasionally associated with other diseases, such as diabetes and cirrhosis[13].

Carnitine deficiency in humans is associated with myopathy[14] and impaired fatty acid oxidation[15]. Coenzyme Q_{10} (CoQ_{10}) or ubiquinone is a vitamin-like substance that is synthesized in all tissues. CoQ_{10} is the coenzyme for at least three mitochondrial enzymes (complexes I, II and III). The electron and proton transfer functions of the quinine ring are of fundamental importance to all life forms[16].

The role of ubiquinone (CoQ_{10}) as a component of the mitochondrial respiratory chain, and an intracellular antioxidant has gained attention. In vitro study demonstrated that CoQ_{10} protected membrane phospholipids and serum LDL from lipid peroxidative stress[17]. In vivo study reported that CoQ_{10} reduced myocardial ischemia and reperfusion injury induced by oxidative stress

through suppression of the formation of reactive oxygen species[18].

MATERIALS & METHODS

Experimental animals

Eighty male albino rats of 150-200g body weight (El-Nasr Pharmaceutical Company,Cairo, Egypt) were recruited for the present study. Rats were housed in metabolic cages at constant experimental condition of temperature of 25°C with 12 hours light/dark cycle. Animals were fed on rodent chow diet (ADWIC Co., Cairo, Egypt) and allowed free access of drinking water.

Induction of Liver cirrhosis

Liver cirrhosis was induced by s.c. injection of a single dose of CCl_4 (1ml/kg)[19]. L-carnitine (50mg/kg/day) was given i.p for four successive days 24 hours before CCl_4 injection[20] and CoQ_{10} (200 mg/kg) was given as a single i.p dose 24 hours before CCl_4 injection[21]. Animals were sacrificed 24 hours after CCl_4 injection, blood samples were withdrawn and liver tissue samples were homogenized and stored at -20°C.

Blood and Tissue sampling

Blood samples were collected at the end of the study from fasting rats via retro-orbital plexus under diethyl ether anaesthia and bleeding, processed for subsequent determinations of amino transferase enzymes (ALT & AST), total LDH activities, total protein and separation of LDH isoenzymes.

Livers were removed from sacrificed rats. Briefly the liver tissues were homogenized in hexane/propanol (3:2 v/v) and centrifuged. The extract was collected and washed with aqueous sodium sulfate. Supernatant was evaporated and the precipitate was weighed, dissolved in 10 ml hexane and stored at -20°C prior to analysis[22]. Lipid peroxides level (MDA), reduced glutathione, total LDH activities, total protein levels was determined and LDH isoenzymes were separated by gel electrophoresis.

Lipid peroxides level was determined according to the method of ***Ohkawa et al.***[23], reduced glutathione content was determined according to the method described by ***Moron et al.***[24]. Serum and hepatic total protein levels were determined according to the method described by ***Bradford***[25]. Amino transferases (ALT & AST) were determined according to the method described by **Reitman and Frankel**[26], total LDH activities was determined according to the method described by ***Babson and Babson***[27] and separation of LDH isoenzymes by gel electrophoresis by the method of ***Dietz and Lubrano***[28].

Statistical analysis

Data were expressed as mean ± SE. Results were analyzed by student -t- test using InStat-3 program. $P<0.05$ was considered statistically significant.

Table (1) : Effect of L-Carnitine or/ and CoQ_{10} treatment on serum ALT, AST, and LDH activities as well as Total Protein levels.

Groups / Parameter	Normal Groups				Cirrhotic Groups			
	Control (I)	L-Carnitine (II)	CoQ_{10} (III)	L-Carnitine + CoQ_{10} (IV)	Control (V)	L-Carnitine (VI)	CoQ_{10} (VII)	L-Carnitine + CoQ_{10} (VIII)
ALT (IU/L)	29.32 ± 2.56	27.17 ± 3.22	16.39 (*) ± 4.82	26.37 ± 3.91	37.49 (*) ± 4.68	29.59 (#) ± 0.69	21.03 (#) ± 1.87	21.05 (#) ± 1.15
AST (IU/L)	71.18 ± 5.15	62.37 (*) ± 3.07	56.44 (*) ± 3.43	59.84 (*) ± 5.40	109.82 (*) ± 7.86	90.82 (#) ± 7.65	87.65 (#) ± 6.06	84.08 (#) ± 3.37
Serum Total Protein (mg/ml)	19.30 ± 0.42	17.32 ± 1.26	20.80 ± 0.91	20.80 ± 0.59	5.50 (*) ± 0.14	14.07 (#) ± 1.33	18.10 (#) ± 0.26	18.53 (#) ± 2.00
Serum LDH (μ/L)	81.42 ± 13.47	77.00 (*) ± 11.76	49.15 (*) ± 14.80	91.14 (*) ± 11.04	209.6 (*) ± 3.94	164.8 (#) ± 10.69	150.7 (#) ± 1.25	128.3 (#) ± 2.04

* Significantly different from group I at $P < 0.05$.
# Significantly different from group V at $P < 0.05$.

Table (2): Effect of L-Carnitine or/ and CoQ_{10} treatment on hepatic MDA, GSH, total protein and LDH

Groups / Parameter	Normal Groups				Cirrhotic Groups			
	Control (I)	L-Carnitine (II)	CoQ_{10} (III)	L-Carnitine + CoQ_{10} (IV)	Control (V)	L-Carnitine (VI)	CoQ_{10} (VII)	L-Carnitine + CoQ_{10} (VIII)
MDA (nmol/mg)	2.57± 0.12	2.25 (*)± 0.18	2.34± 0.32	1.68 (*)± 0.26	5.98 (*)± 0.27	3.77 (#)± 0.12	3.48 (#)± 0.16	3.55 (#)± 0.11
GSH (mg/g tissue)	3.93± 0.07	5.33 (*)± 0.88	6.19 (*)± 0.92	5.81 (*)± 1.00	2.51 (*)± 0.40	3.64 (#)± 0.20	3.57 (#)± 0.85	3.67 (#)± 0.41
Hepatic Total Protein (mg/g tissue)	250.11 ± 23.58	258.28± 35.71	256.05 ± 8.83	287.96 (*) ± 35.36	176.48 (*)± 6.23	217.24 (#) ± 14.96	232.99 (#)± 20.36	248.00 (#)± 19.06
Hepatic LDH (µ/L)	114.0± 1.05	88.9 (*) ± 0.15	99.57 (*)± 4.43	97.6 (*)± 1.05	181.9 (*)± 1.71	163.9 (#)± 5.73	155.3 (#)± 1.46	134.9 (#)± 5.48

* *Significantly different from group I at $P < 0.05$.*
\# *Significantly different from group V at $P < 0.05$.*

Separation of serum LDH isoenzymes by gel electrophoresis:

Figure (2), tables (3) and (4) show the electrophoretic separation pattern of serum lactate dehydrogenase isoenzymes in normal and cirrhotic groups. It can be observed that CCl_4 injection resulted in a significant increase in serum levels of all LDH isoenzymes. Administration of L-carnitine alone significantly decreased serum LDH-1 and LDH-5; however, it increased serum LDH-2, LDH-3 and LDH-4 compared to CCl_4 control group, administration of coenzyme Q_{10} alone significantly decreased serum levels of all LDH isoenzymes compared to CCl_4 control group. On the other hand, administration of a combination of L-carnitine and coenzyme Q_{10} significantly decreased serum LDH-1, LDH-2, LDH-3 and LDH-5; however, it increased serum LDH-4 compared to CCl_4 control group.

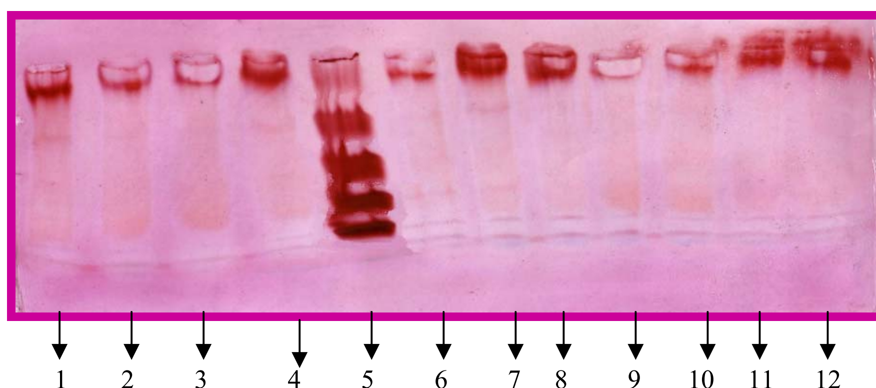

Fig. (1): Electrophoretic Profile of Serum Lactate Dehydrogenase Isoenzymes (LDH- Isoenzymes) of Male Rats.

Lane 1	:	Normal Control Group
Lanes 2, 3	:	L-Carnitine Group
Lane 4	:	CoQ_{10} Group
Lane 5	:	Heart (Standard Group)
Lane 6	:	L-Carnitine & CoQ_{10} Group
Lane 7	:	CCl_4 Control Group
Lane 8	:	L-Carnitine & CCl_4 Group
Lanes 9, 10	:	CoQ_{10} & CCl_4 Group
Lanes11, 12	:	L-Carnitine, CoQ_{10} & CCl_4 Group

Table (3): % serum LDH isoenzyme pattern in all groups

Groups / % Serum LDH Isoenzymes	Normal Groups				Cirrhotic Groups			
	Control (I)	L-Carnitine (II)	CoQ_{10} (III)	L-Carnitine + CoQ_{10} (IV)	Control (V)	L-Carnitine (VI)	CoQ_{10} (VII)	L-Carnitine + CoQ_{10} (VIII)
% LDH-1	1.63 ± 0.102	2.25±0.717	1.68±0.664	3.45±0.476	3.12±0.993	2.11±0.310	2.56±0.608	2.04±0.614
% LDH-2	2.10±0.287	2.10±0.0637	2.35±0.339	4.08±0.194	3.63±0.453	5.37±0.158	2.98±0.793	3.11±0.505
% LDH-3	4.15±0.209	5.75±0.288	2.46±0.265	4.02±0.103	2.34±0.253	5.39±0.078	2.54±0.556	2.65±0.505
% LDH-4	5.22±0.204	8.61±2.278	6.25±0.187	6.13±0.206	2.61±0.251	5.62±0.074	3.54±1.362	4.45±1.338
% LDH-5	86.88±0.172	81.27±3.296	87.25±0.367	82.31±0.386	88.29±1.221	81.51±0.385	88.36±2.441	87.40±1.951

Table (4): Activity of Serum LDH Isoenzymes (μ/L)

Groups / Serum LDH Isoenzymes (μ/L)	Normal Groups				Cirrhotic Groups			
	Control (I)	L-Carnitine (II)	CoQ_{10} (III)	L-Carnitine + CoQ_{10} (IV)	Control (V)	L-Carnitine (VI)	CoQ_{10} (VII)	L-Carnitine + CoQ_{10} (VIII)
LDH-1	1.33	1.73 (*)	0.82 (*)	3.14 (*)	6.55 (*)	3.46 (#)	3.86 (#)	3.05 (#)
LDH-2	1.71	1.62 (*)	1.15 (*)	3.72 (*)	7.60 (*)	8.85 (#)	4.49 (#)	3.97 (#)
LDH-3	3.38	4.43 (*)	1.21 (*)	3.66 (*)	4.89 (*)	8.88 (#)	3.83 (#)	3.39 (#)
LDH-4	4.25	6.63 (*)	3.07 (*)	5.59 (*)	5.48 (*)	9.26 (#)	5.35 (#)	5.10 (#)
LDH-5	70.74	62.58 (*)	42.88 (*)	75.02 (*)	185.06 (*)	134.33 (#)	133.15(#)	111.88 (#)

* Significantly different from group I at $P < 0.05$
# Significantly different from group V at $P < 0.05$

DISCUSSION

Cirrhosis is an irreversible result of various disorders that damage liver cells over time with no proven effective therapy. Eventually, damage becomes so extensive that the normal structure of the liver is distorted and its function is impaired[(29)].

Reactive oxygen species (ROS) as free radicals can initiate lipid peroxidation and DNA damage leading to cell death, if the antioxidant system is impaired. Oxygen derived free radicals are continuously generated in the cells[(30)]. The production of ROS in the biological system results from the sequential univalent reduction of molecular oxygen[(31)], leading to the formation of superoxide radical ($O_2^{\bullet -}$). Once formed, which undergoes a variety of chemical reactions yielding other ROS like hydroperoxyl radical ($HO_2^{\bullet}$). Reaction between $O_2^{\bullet -}$ and H_2O_2 in the presence of certain transition metals such as iron can yield the potent oxidizing agent ($OH^{\bullet}$)[(32)].

Oxidative stress has a role in liver injury, cirrhosis development and carcinogenesis[(33)]. Mitochondria is the main generator of superoxide in hepatocytes. The mechanism of superoxide production is linked to either the disorder in the operation of the ubiquinone (CoQ_{10}) cycle (complex III)[(34)] and/or reducing equivalents which can not be transferred to O_2 at the mitochondrial cytochrome-c oxidase, due to oxygen deficiency[(35)].

Carbon tetrachloride injection is used to provide animal model of liver damage which is caused by formation of trichloromethyl and trichloromethylperoxyl radicals, initiating lipid peroxidation and resulting in fibrosis and cell necrosis[(36)]. The oxidative stress in rat liver and lipid peroxidation caused by administration of CCl_4 has been reported[(37,38)].

Carbon tetrachloride treated rats showed a significant increase of plasma activities of aminotransferases (ALT and AST), malondialdehyde (MDA) formation confirming other study[(39,40,41,42)], a significant decrease in hepatic total protein, this result confirmed previous result[(39)]. Also, it showed decreased serum level of total protein confirming other result[(43)] and hepatic reduced glutathione a result is in agreement with that of **Allis et al**.[(38)]. The earliest change in CCl_4 hepatotoxicity is the blockage of lipoprotein secretion and the accumulation of lipids in the liver[(44)].

In the present investigation, CCl_4 injection produced a significant increase in serum total LDH activity finding confirming other studies[(39,41)] and caused a significant increase in serum levels of all LDH isoenzymes. Also, there was a significant increase in hepatic LDH activity after injection of CCl_4, in accordance with the result of **Seeto et al.**[(45)], this is due to the increasing in LDH leakage from rat hepatocytes that may be attributed to CCl_4-induced dehalogenation in the liver endoplasmic reticulum. This process leads to trichlormethyl radical ($CCl_3^{\bullet}$) formation and initiation of lipid peroxidation[(46)].

In the present study, levels of MDA were shown to be significantly decreased on injection of L-carnitine in CCl_4 induced hepatotoxicity. L-carnitine administration stimulates β-

oxidation of fatty acids and reduces the esterification of triacylglycerol[47] and its level in the liver tissue was found to be low in the case of cirrhosis[48]. L-carnitine may lead to the inhibition of lipid peroxidation by enhancing antioxidant capacity[49].

The CoQ_{10} was accompanied by a marked reduction of lipid peroxides level and normalization of reduced glutathione levels in liver tissue of rats injected with CCl_4. These results confirm the beneficial antioxidant activity of ubiquinone[50,18,51]. Administration of ubiquinone significantly prevented CCl_4 induced oxidative stress and lipid peroxidation in rat liver[37,38] because the coenzyme Q_{10} increases the activity of the electron transport chain both in vitro and in vivo[52], beside its antioxidant effects[53,54].

Administration of L-carnitine or/and coenzyme Q_{10} (prior to CCl_4 injection) significantly increased hepatic and serum total protein, hepatic reduced glutathione content and significantly decreased serum ALT and AST activities compared to CCl_4 control group. Marked improvement has been achieved by the combination of L-carnitine and coenzyme Q_{10} in agreement with the result of **Wang et al.**[42].

Administration of L-carnitine or/and coenzyme Q_{10} (prior to CCl_4 injection) significantly decreased hepatic and serum total LDH activity compared to CCl_4 control group in harmony with other studies[39,42].

LDH-1 isoenzyme is maximally active at low concentration of pyruvate and inhibited by excess pyruvate, while LDH-5 isoenzyme maintains its activity at high pyruvate concentration[55]. The electrophoretic separation pattern of serum lactate dehydrogenase isoenzymes in normal and cirrhotic groups showed that CCl_4 injection resulted in a significant increase in serum levels of all LDH isoenzymes. The treatment with L-carnitine resulted in a significant increase in isoenzyme 2, 3 and 4; and a significant decrease in isoenzyme 1 and 5. Treatment with coenzyme Q_{10} resulted in a significant decrease in all isoenzymes. Treatment with a combination of L-carnitine and coenzyme Q_{10} resulted in a significant increase in isoenzyme 4 and a significant decrease in isoenzyme 1, 2, 3 and 5.

Conclusion: From the previous results, it could be concluded that L-carnitine and coenzyme Q_{10} have a pronounced prophylactic effect against liver damage induced by halogenated alkanes and the combination of L-carnitine and CoQ_{10}.

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"دور بيتا- هيدروكسي- جاما- ثلاثي ميثيل الأمونيوم بيوتيرات و يوبيكينون في تلاشي التأثير الضار للألكانات الهالوجينية على الكبد "

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ان تليف الكبد من أخطر المشاكل الصحية الناتجة عن الكثير من الأضرار التي تدمر خلايا الكبد بمرور الوقت.
وهذا البحث يهدف الى تقديم معلومات عن التأثير الضار للألكانات الهالوجينية (رابع كلوريد الكربون) على خلايا الكبد وتقييم دور كل من إل- كارنيتين و مساعد الإنزيم كيو ١٠ في الوقاية من هذا التأثير الضار.
أجريت هذه الدراسة على ٨٠ من ذكور الجرذان البيضاء البالغة حيث قسمت الجرذان إلى ثماني مجموعات (بكل مجموعة ١٠ جرذان) كالآتي: أربعة مجموعات طبيعية (مجموعة ضابطة، مجموعة حقنت بلإل- كارنيتين، مجموعة حقنت بمساعد الإنزيم كيو١٠ و مجموعة حقنت بكلاهما) و أربعة مجموعات استحدث بها التليف الكبدي (مجموعة ضابطة، مجموعة حقنت بلإل-كارنيتين، مجموعة حقنت بمساعد الإنزيم كيو١٠ ومجموعة حقنت بكلاهما). وقد أستحدث التليف الكبدي بحقن الجرذان بجرعة واحدة من رابع كلوريد الكربون (١ مل/كجم) تحت الجلد. بينما تم الحقن البريتوني لإل- كارنيتين (٥٠ مجم/كجم/يوم) لمدة ٤ أيام متتالية و جرعة واحدة من مساعد الإنزيم كيو١٠ (٢٠٠ مجم/كجم) قبل حقن رابع كلوريد الكربون ب ٢٤ ساعة.
وتم ذبح جميع الجرذان بعد حقن رابع كلوريد الكربون ب٢٤ ساعة، وسحبت عينات الدم لفصل مصل الدم ، وفصل الكبد وعمل خليط متجانس منه وتم حفظهما لحين الاستعمال. وقد تم قياس كل من المحتوى الكبدي للجلوتاثيون المختزل ، نشاط الإنزيمات الناقلة للأمين في مصل الدم ، المحتوى الكبدي للأكسيدات الفوقية للدهون، محتوى البروتين الكلي في مصل الدم والكبد ، مستوى إنزيم اللاكتات ديهيدروجينيز في مصل الدم والكبد و مستوى النظائر المختلفة لإنزيم اللاكتات ديهيدروجينيز الكلي في مصل الدم باستخدام طريقة الفصل الكهربي.
وكانت نتائج الدراسة أن حقن رابع كلوريد الكربون يؤدي إلى انخفاض ذي دلالة إحصائية في المحتوى الكبدي للجلوتاثيون المختزل ، البروتين الكلي في الكبد ومصل الدم ، كما يؤدي إلى ارتفاع ذي دلالة إحصائية في نشاط الإنزيمات الناقلة للأمين في مصل الدم، والمحتوى الكبدي للأكسيدات الفوقية للدهون، مستوى إنزيم اللاكتات ديهيدروجينيز في مصل الدم والكبد و يؤدي إلى ارتفاع ذي دلالة إحصائية في مستوى النظائر المختلفة لإنزيم اللاكتات ديهيدروجينيز في مصل الدم. ومن ناحية أخرى فقد ثبت أن الحقن البريتوني لإل- كارنيتين أو مساعد الإنزيم كيو١٠ أو كلاهما معا قبل حقن رابع كلوريد الكربون يؤدي إلى تحسن ملحوظ في معظم المعايير التي تم قياسها بالمقارنة بالمجموعة المريضة الضابطة.
لذا فقد أوضحت هذه الدراسة الدور الوقائي لإل- كارنيتين ومساعد الإنزيم كيو١٠ كمضادات للأكسدة تحمي خلايا الكبد من التلف الناتج عن اللألكانات الهالوجينية.